

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07F 9/02	A1	(11) International Publication Number: WO 96/15132 (43) International Publication Date: 23 May 1996 (23.05.96)
(21) International Application Number: PCT/US95/14940 (22) International Filing Date: 15 November 1995 (15.11.95) (30) Priority Data: 08/340,161 15 November 1994 (15.11.94) US (60) Parent Application or Grant (63) Related by Continuation US 08/340,161 (CIP) Filed on 15 November 1994 (15.11.94) (71) Applicant (for all designated States except US): THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 22nd floor, 300 Lakeside Drive, Oakland, CA 94612-3550 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): HOSTETLER, Karl, Y. [US/US]; 22nd floor, 300 Lakeside Drive, Oakland, CA 94612-3550 (US). KINI, Ganesh, D., Ph., D. [US/US]; 22nd floor, 300 Lakeside Drive, Oakland, CA 94612-3550 (US).	(74) Agent: ALTMAN, Daniel, E.; Knobbe, Martens, Olson & Bear, 16th floor, 620 Newport Center Drive, Newport Beach, CA 92660 (US). (81) Designated States: AL, AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, LS, MW, SD, SZ, UG). Published With international search report.	

(54) Title: **IMPROVED ANTIVIRAL PRODRUGS**

(57) Abstract

Lipid prodrugs of phosphonoacids and their analogs that have increased antiviral activity over the parent drugs in inhibiting cytomegalovirus and other susceptible viruses.

BEST AVAILABLE COPY

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LJ	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

IMPROVED ANTIVIRAL PRODRUGS

5 The present invention relates generally to lipid derivatives of antiviral agents. It relates particularly to lipid prodrugs of phosphonoacids and their use in the treatment of viral infections.

10 Phosphonoacetate and phosphonoformate were first synthesized in 1924 (Nylén, Chem. Berichte 57:1023); however, the ability of these compounds to inhibit viral enzymes selectively was not immediately demonstrated. Helgstrand, et al. Science 201:819-821 (September 1, 1978) disclosed that both phosphonoacetic acid and phosphonoformic acid inhibit several DNA polymerases and preferentially inhibit several viral DNA polymerases. Phosphonoformate and phosphonoacetate are presently known to selectively inhibit the DNA polymerase of many viruses, including human cytomegalovirus (HCMV), herpes simplex virus (HSV) and the reverse transcriptase of human immunodeficiency virus (HIV). Chrisp and Clissold (1991) Drugs 41:104 review the pharmacology of these agents. 20 Phosphonoacetate is too toxic for use in humans, but phosphonoformate (Foscavir, Astra) is approved for human use in HCMV-infected AIDS patients. However, it is not highly potent, requires prolonged intravenous administration and has substantial toxicity to the kidney and other organs. 25 Ericksson, et al., U.S. Patents Nos. 4,215,113; 4,339,445; 4,665,062; 4,771,041 teach the use of phosphonoformic acid as the selective agent in treating viral infections, including herpes virus type I and II and cytomegalovirus, in treating cancer caused by virus, and also opposing transformation of 30 cells caused by oncogenic viruses.

Derivatized forms of phosphonoacids and pharmaceutical formulations comprising these compounds are known. U.S. Patent No. 5,072,032 to McKenna discloses thiophosphonoacids; 35 Nos. 4,386,081 and 4,591,583 to Helgstrand et al. disclose phosphonoformic acid esters of alkyl, alkylene, alkoxy and related cyclic and aromatic groups and some of these are shown

to inhibit herpes virus and the functions and intracellular multiplication of influenza virus. U.S. Patent No. 5,194,654 to Hostetler et al, discloses phospholipid derivatives of phosphonoacids, their incorporation into liposomes and their use as selective antiviral and antiretroviral agents.

There is a continuing need for less toxic, more selective and more effective antiviral prodrugs of the phosphonoacids.

SUMMARY OF THE INVENTION

According to the invention there are provided compounds having the structure [I] and the substituents R₁, R₂, R₃, Y, Z, A, X, m, and n as defined herein. According to a preferred embodiment of the invention, m is 0. In yet another preferred embodiment X is oxygen.

According to other preferred embodiments of the invention, R₁ is an O-alkyl group; particularly preferred are compounds wherein R₁ is an O-octadecyl group. Also preferred are compounds wherein R₂ is an O-benzyl group or an OCH₃ group.

In yet another preferred embodiment, Z₁ and Z₂ are independently R₃ and R₃ is a methyl group. Particularly preferred compounds are 1-O-octadecyl-1,3-propanediol-3-phosphonoacids; 1-O-octadecyl-1,3-propanediol-3-thiophosphonoacids; 1-O-octadecyl-2-O-methyl-sn-glycero-3-phosphonoacids; 1-O-octadecyl-2-O-methyl-sn-glycero-3-thiophosphonoacids; 1-O-octadecyl-2-O-benzyl-sn-glycero-3-phosphonoacids; 1-O-octadecyl-2-O-benzyl-sn-glycero-3-thiophosphonoacids. 1-O-octadecyl-sn-glycero-3-phosphonoacid ethyl esters; 1-O-octadecyl-sn-glycero-3-thiophosphonoacid ethyl esters; 1,2-dimyristoyl-sn-glycero-3-phosphonoacid ethyl esters; 1,2-dimyristoyl-sn-glycero-3-thiophosphonoacid ethyl esters;

According to another aspect of the invention there are provided compounds having the general structure of Formula [II] wherein R₁, R₂, R₃, Y, Z, A, m and n are as defined herein. Preferred compounds among this group are 1-O-

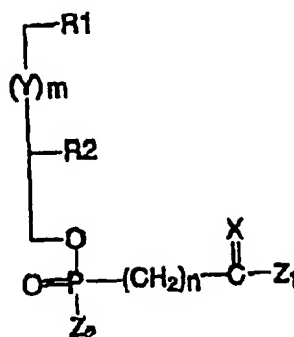
octadecyl-sn-glycero-3-phosphonoacetates; and 1-O-octadecyl-2-O-benzyl-sn-glycero-3-phosphonoformates.

According to yet another aspect of the invention there are provided 2-carbon analogues of the compounds of the invention having the structure of formula III or formula IV. Preferred compounds according to this embodiment are 1-O-octadecyl-1,2-ethanediol-2-phosphonoacetate; 1-O-octadecyl-1,2-ethanediol-2-phosphonoformate; 1-O-octadecyl-1,2,ethanediol-(C)-phosphonoformate; and 1-O-octadecyl-1,2,ethanediol-(C)-phosphonoacetate.

According to yet another aspect of the invention there are provided liposomes or other lipid vesicles formed in part from a compound according to the invention. The invention also provides methods for treating a viral or retroviral infection in a mammal, comprising administering an effective amount of a compound according to the invention to the mammal. These methods can further comprise the co-administration of an antiviral nucleoside analogue, a viral protease inhibitor or other antiviral agent.

It is an object of the invention to provide lipid prodrugs of the phosphonoacids that retain the pharmacological effects of the parent compounds. Unexpectedly, it has been found that the compounds of the present invention have advantageous pharmacological effects over previously known prodrugs of this type. The invention accordingly provides a series of improved prodrugs of phosphonoformate and phosphonoacetate and their analogs having substantial increases in antiviral activity over the parent compounds against human cytomegalovirus (HCMV), herpes simplex virus (HSV), and human immunodeficiency virus (HIV-1). This enhanced antiviral activity can be demonstrated in cell culture, for example, by means of the in vitro susceptibility assays described in Examples 17 through 20.

The compounds of the invention have the general formula [I]:



wherein

R1 is O-Alkyl C₁ to C₂₄, 0-6 double bonds;

S-Alkyl C₁ to C₂₄, 0-6 double bonds;

O-Acyl C₁ to C₂₄, 0-6 double bonds; or

S-Acyl C₁ to C₂₄, 0-6 double bonds;

each R2 is independently OH, H, a halogen such as fluorine, chlorine, iodine and bromine, OCH₃, O-benzyl, SH, SCH₃, or NH₂;

O-Acyl C₁ to C₂₄, 0-6 double bonds;

S-Acyl C₁ to C₂₄, 0-6 double bonds;

O-Alkyl C₁ to C₂₄, 0-6 double bonds;

S-Alkyl C₁ to C₂₄, 0-6 double bonds;

N-Acyl C₁ to C₂₄, 0-6 double bonds; or

N-Alkyl or N-dialkyl, C₁ to C₂₄, 0-6 double bonds;

Z is R3 when the compound of formula [I] is esterified or A' when [I] is in the form of a salt or a combination thereof;

R3 is independently selected from the group of alcohols consisting of substituted or unsubstituted linear C₁ to C₆ alkyl groups, substituted or unsubstituted branched C₃ to C₆ alkyl groups, CH₃(CH₂)_nNH₂, wherein n is 0 to 8,

(CH₃)₃N⁺CH₂CH₂OH, HOCH₂CH₂NH₂, HOCH₂CH(NH₂)CO₂H, C₆H₁₂O₆,

CH₂OHCHOHCH₂OH; CH₂Ph; pentoses, hexoses and their corresponding alcohols;

Each A' is independently selected from the group consisting of H⁺, Na⁺, NH₄⁺; amines selected from the group consisting of mono-, di-, and trialkylamines; and other physiologically

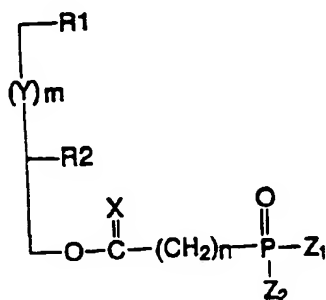
acceptable cations; and n = 0 or 1.

Pharm. Sci. 66(1):1-19. Preferred cations are sodium or potassium ions.

Y is CH-R₂; m = 0-6; and when m ≥ 1, Y is a CH-R₂ group, wherein R₂ is independently selected from the group defined above; X is O, S, or Se; and
 5 n is 0 or 1.

The alkyl and acyl groups of R₁, and R₂ can be substituted or unsubstituted, linear or branched. The result of the independent selection of R₂ groups on each of the
 10 internal Y carbons when m > 0 is that the molecule may comprise from 2 to 7 different R₂ groups.

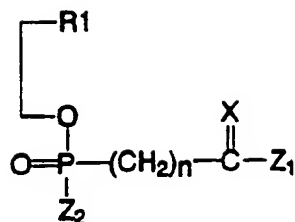
Alternatively, the compounds of the invention are of the general formula [II]:



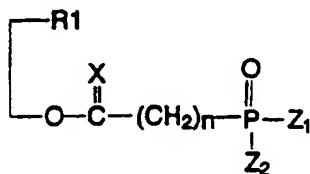
30 wherein R₁, R₂, R₃, Y, Z₁, Z₂ and A*, X, m and n are defined as above. The compounds of formula II differ from those of formula I by having the phosphonoacid moiety coupled to the lipid moiety through a carboxy rather than a phosphate linkage. The invention also include lipid derivatives of
 35 phosphonoacids that are substituted ethanediols of the general formula [III]:

40

45



wherein R1, R2, R3, Y, Z₁, Z₂, X, m and n are as defined above. These ethanediol-based species can be linked to the phosphonoacids through a carbon linkage, and these compounds are of the general formula [IV]:



wherein R1, R2, R3, Y, Z₁, Z₂, X, m and n are as defined above.

Synthesis of improved prodrugs of the phosphonoacids

The compounds that are synthesized are outlined in Scheme I with the various substituents on C1, C2 and C3 of the glycerol, and similarly in Schemes II and III for compounds wherein (Y)_m is (CH-R2)_m and m ≥ 1.

Identification of Starting Materials and Products

The phosphonoacids to which various lipid moieties are coupled in the preparation of the lipid prodrugs of the invention are designated by acronyms, as follows:

- n = 0, X = O (PFA) Phosphonoformic acid
n = 0, X = S (PFSA) Thiophosphonoformic acid
n = 0, X = Se (PFSeA) Selenophosphonoformic acid
n = 1, X = O (PAA) Phosphonoacetic acid
5 n = 1, X = S (PASA) Thiophosphonoacetic acid
n = 1, X = Se (PASEA) Selenophosphonoacetic acid

The various lipid prodrug derivatives are designated herein by acronyms derived from those above, and defined in the legends of Tables I-IV.

10 Synthesis Procedures

Lipid prodrugs of the phosphonoacids listed above are prepared according to the procedures described in Examples 1-16. A synthesis flow diagram particularly relevant to the synthesis of compounds wherein m = 0 and Y is absent is set forth in Scheme I; flow diagrams particularly relevant to the chemical synthesis of compounds wherein m > 0 and Y is present are set forth in Schemes II and III.

15 Antiviral Activity

The antiviral activity of various lipid derivatives of phosphonoacids according to the invention was determined in cultures of human cell lines infected with HCMV, HSV, or HIV-1 as described in Example 17. The results are shown in Tables I-IV. The predictive value of in vitro susceptibility testing for viral infections is discussed by Kern, ER (1990) Preclinical evaluation of antiviral agents: In vitro and animal model testing. Galasso, G. et al. eds. Antiviral Agents and Viral Disease of Man, 3rd Edition, Raven Press, NY pp. 87-123.

The most preferred compounds which exhibit greatly increased antiviral activity (Tables I-IV) have 1-O-alkyl groups at R1, and hydroxyl, hydrogen, O-methyl or O-benzyl at R2.

Antiviral activity of the improved phosphonoacid prodrugs against human cytomegalovirus-infected MRC5 human lung fibroblast cells is shown in Tables I and II. the most preferred prodrugs of phosphonoformate (Table I) have remarkable increases in antiviral activity. Previous attempts

fibroblast cells is shown in Tables I and II. the most preferred prodrugs of phosphonoformate (Table I) have remarkable increases in antiviral activity. Previous attempts to produce prodrugs of phosphonoformate with increased activity have identified a few compounds which have very small increases in activity, but no compound having increases in activity over PFA greater than 1.9 fold have been shown previously (Norén, J.O., et al., J. Med. Chem., 26:264-270, 1983). The most active PFA prodrugs, B-PFA, BB-PFA, and MB-PFA, exhibit 107-, 72- and 38-fold increases in activity. The 3B-PFA stereoisomer and (rac) B-PFA, a mixture of the stereoisomers, were essentially equivalent to B-PFA indicating that both stereoisomers of glycerol have a very high degree of antiviral activity. A series of analogs having hydrogen at R2 and 16 to 22 carbon alkyl (oxy) ethers at R1 were also highly active with 51- to 124-fold increases in activity over PFA. The most active of these was 1-O-oleylpropanediol-3-PFA which inhibited HCMV replication by 50% at 0.37 μ M. The compounds noted above represent the most active PFA-containing compounds yet reported. These compounds have a 1-O-alkyl group at the R1 position of glycerol and either a hydroxyl, -O-benzyl or -O-methyl or hydrogen moiety at the R2 position. Prodrugs having H, halogen or amino at R2 will also be highly active and substitution at X of S or Se for O will provide similar results.

BB-PFA-OEt and B-PFA-OMe retains substantial activity (16-fold increase over PFA) with a carboxyethyl or carboxymethyl ester at the R3 position. Other ester substituents at R3 which will provide excellent activity include benzyl, choline, ethanolamine, glycerol and inositol. These compounds may be converted to active drugs inside the target cells more readily than carboxyethyl esters.

Similar results were obtained in human cytomegalovirus-infected cells with the phosphonoacetate series of compounds shown in Table II. Here the order of activity was slightly different with B-PAA, MB-PAA and BB-PAA exhibit 100-, 36- and 24-fold increases in activity compared with free PAA. When

the phosphonoacetic acid is linked to glycerol at the sn-3 hydroxyl in a carboxyester linkage as in BB-(C)-PAA, nearly full antiviral activity is retained (16-fold increase vs PAA). Glycerol-PAA derivatives with two acyl chains (DMG-PAA), or two phosphorus residues and two acyl esters (DMP-PAA and DPP-PAA), did not exhibit substantially increased activity (1.2-, 0.24- and 0.28-times the activity of PAA, respectively).

The improved PFA prodrugs also exhibit greatly increased activity versus PFA in herpes simplex virus-1 infected human lung fibroblasts (Table III). MB-PFA, B-PFA and BB-PFA are 72-, 43- and 34-times more active than PFA and represent the most active PFA derivatives yet reported. The order of activity is slightly different from that observed with human cytomegalovirus; MB-PFA is the most active compound followed by B-PFA and BB-PFA. Similar results were obtained with human immunodeficiency virus-1 infected cells in vitro (Table IV). With HIV-1, MB-PFA and 3B-PFA were the most active compounds followed by (rac)B-PFA, thio-ODG-PFA, thio-HDG-PFA and B-PFA; the compounds were 222-, 166-, 102-, 57-, 48- and 37-fold more active than PFA in HIV-infected HT4-6C cells and represent the most active anti-HIV derivatives of PFA reported. MB-PFA was more active than B-PFA to a statistically significant degree (Table IV).

Therapy of Viral Diseases

The lipid derivatives of phosphonoacids disclosed herein are useful in treating diseases caused by viruses such as influenza, herpes simplex virus (HSV), human herpes virus 6, cytomegalovirus (CMV), hepatitis B virus, Epstein-Barr virus (EBV), and varicella zoster virus (VZV). They are useful in the treatment of AIDS and other retroviral diseases, as well.

Lipid derivatives of antiviral phosphonoacids may be applied topically to the skin, eyes or mucous membranes or into the interior of the body, for treating susceptible virus infections in man and animals. They can be introduced internally, for example orally, intratracheally or otherwise by the pulmonary route, enterally, rectally, nasally, vaginally, lingually, intravenously, intra-arterially,

intramuscularly, intraperitoneally, intradermally, or subcutaneously. The present pharmaceutical preparations can contain the active agent alone, or can contain further pharmaceutically valuable substances. For example, formulations comprising lipid phosphonoacid prodrugs of the invention can additionally comprise another antiviral agent, such as for example, a viral protease inhibitor, or an antiviral nucleoside analogue. They can further comprise a pharmaceutically acceptable carrier.

Lipid derivatives of antiviral agents may have a prolonged antiviral effect as compared to the lipid-free agents; therefore they provide therapeutic advantages as medicaments even when not incorporated into liposomes. These phosphonoacid prodrug antiviral agents may be used alone or in combination with antiviral nucleosides as given conventionally. The use of combination therapy may greatly reduce the tendency for drug resistant HIV mutant strains to appear and would therefore increase the likelihood of stopping the progression of HIV infection. The same argument would hold equally well in treating cytomegalovirus or herpes virus infections with regard to the likelihood of developing resistant strains.

Formulations

Pharmaceutical preparations containing lipid derivatives of antiviral phosphonoacids are produced by conventional dissolving and lyophilizing processes to contain from approximately 0.1% to 100%, preferably from approximately 1% to 90% of the active ingredient. They can be prepared as ointments, salves, tablets, capsules, powders or sprays, together with effective excipients, vehicles, diluents, fragrances or flavor to make palatable or pleasing to use.

Formulations for oral ingestion are in the form of tablets, capsules, pills, ampules of powdered active agent, or oily or aqueous suspensions or solutions. Tablets or other non-liquid oral compositions may contain acceptable excipients, known to the art for the manufacture of pharmaceutical compositions, comprising diluents, such as

lactose or calcium carbonate; binding agents such as gelatin or starch; and one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring or preserving agents to provide a palatable preparation. Moreover, such oral preparations may be coated by known techniques to further delay disintegration and absorption in the intestinal tract. The preparations can also comprise bile salts and detergents.

Aqueous suspensions may contain the active ingredient in admixture with pharmacologically acceptable excipients, comprising suspending agents, such as methyl cellulose; and wetting agents, such as lecithin, lysolethicin or long-chain fatty alcohols. The said aqueous suspensions may also contain preservatives, coloring agents, flavoring agents and sweetening agents in accordance with industry standards.

Preparations for topical and local application comprise aerosol sprays, lotions, gels and ointments in pharmaceutically appropriate vehicles which may comprise lower aliphatic alcohols, polyglycols such as glycerol, polyethylene glycol, esters of fatty acids, oils and fats, and silicones. The preparations may further comprise antioxidants, such as ascorbic acid or tocopherol, and preservatives, such as p-hydroxybenzoic acid esters.

Parenteral preparations comprise particularly sterile or sterilized products. Injectable compositions may be provided containing the active compound and any of the well known injectable carriers. These may contain salts for regulating the osmotic pressure.

Liposomal Formulations

If desired, the compounds can be incorporated into liposomes by any of the reported methods of preparing liposomes for use in treating viral diseases such as, but not limited to HCMV, HSV, and HIV-1. The present invention may utilize the antiviral phosphonoacid derivatives noted above incorporated in liposomes in order to direct these compounds to macrophages, monocytes, other cells and tissues and organs which take up the liposomal composition. The liposome-

incorporated phosphonoacid derivatives of the invention can be used to treat HCMV, HSV or AIDS patients by parenteral administration, enhancing delivery of the antiviral compound to macrophages and monocytes, an important reservoir of viral infections. This will allow for the efficacious use of lower doses of the modified phosphonoacids, reducing toxicity of the compound. Ligands may also be incorporated to further focus the specificity of the liposomes.

The derivatives described have several unique and novel advantages over the liposomal water soluble phosphonoformates. First, they can be formulated in liposomes to much higher ratios of drug to lipid because they are incorporated into the wall of the liposome instead of being located in the aqueous core compartment. Secondly, the liposomes containing the lipophilic phosphonoformate derivatives noted above do not leak during storage, providing improved product stability. Furthermore, these compositions may be lyophilized, stored dry at room temperature, and reconstituted for use, providing improved shelf life. They also permit efficient incorporation of antiviral compounds into liposomal formulations without significant waste of active compound. A further advantage is that the compositions used in vivo treatment cause a larger percentage of the administered antiviral lipid-phosphonoacid conjugate to reach the intended target. At the same time the use of the compositions reduces the amount being taken up by the kidney and bone, thereby decreasing the toxic side effects of the phosphonoacid drug. The toxic side effects of the phosphonoformates may be further reduced by targeting the liposomes in which they are contained to actual or potential sites of infection by incorporating ligands into the liposomes. The liposome-incorporated lipid-phosphonoacid conjugate is administered to patients by any of the known procedures utilized for administering liposomes. The liposomes can be administered intravenously, intraperitoneally, intramuscularly, intravitreally or subcutaneously as a buffered aqueous solution. Any pharmaceutically acceptable aqueous buffer or other vehicle

may be utilized so long as it does not destroy the liposome structure or the activity of the lipid phosphonoacid analogue. One suitable aqueous buffer is isotonic sorbitol containing 5 mM sodium phosphate with a pH of about 7.4, or other physiological buffered salt solutions.

The therapeutically effective amount of the lipid derivatives is determined by reference to the recommended dosages of the active antiviral phosphonoacid, bearing in mind that, in selecting the appropriate dosage in any specific case, consideration must be given to the patient's weight, general health, metabolism, age and other factors which influence response to the drug. The dosage for a mammal, including a human, may vary depending upon the extent and severity of the infection and the activity of the administered compound. Dosage levels of liposomal lipid analogs of phosphonoacids will be about the same as for the phosphonoacid itself. Dosage levels for the phosphonoacids through conventional administration by intravenous infusion are well established (Lambert, R., et al. (1989) J. Med. Chem. 32:367-374; Szoka, F. and Chu, C-J., Antimicrobial Agents and Chemotherapy: 32(6) 858-864 (1988); Ericksson et al. U.S. Patent No. 4,771,041). Foscarnet is administered by i.v. infusion at 200 mg/kg/day for treatment of HCMV in humans.

The phosphonoacid prodrugs of the invention are administered to the patient on a daily basis in an oral dose of about 0.1 mg/kilogram to 1000 mg/kilogram and more preferably from about 1 mg/kilogram to about 400 mg/kilogram. The parenteral dosage will be appropriately 20 to 100% of the oral dose.

Liposome Preparation

After synthesis and purification, the lipid derivative of the phosphonoacid is incorporated into liposomes, or other suitable carrier. The incorporation can be carried out according to well known liposome preparation procedures, such as sonication and extrusion. Suitable conventional methods of liposome preparation include, but are not limited to, those disclosed by Bangham, et al. (Bangham, A.D., Standish, M.M.

and Watkins, J.C. (1965) J. Mol. Biol., 23: 238-252.) Olson, et al. (Olson, F., Hunt, C.A. Szoka, F.C., Vail, W.J. and Papahadjapoulos, D. (1979) Biochim, Biophys. Acta, 557: 9-23.), Szoka, F. and Papahadjapoulos, D. (1978) Proc. Nat. Acad. Sci. 75: 4194-4198, Mayhew, E. et al. (1984) 775: 169-175), Kim, S. et al. (1983) Biochim. Biophys. Act, 728: 339:348, and Mayer, et al. (1986) Biochim. Biophys. Acta, 858: 161-168.

The liposomes may be made from the lipid derivatives of phosphonoacids alone or in combination with any of the conventional synthetic or natural phospholipid liposome materials including phospholipids from natural sources such as egg, plant or animal sources such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, sphingomyelin, phosphatidylserine, or phosphatidylinositol. Synthetic phospholipids that may also be used, include, but are not limited to: dimyristoylphosphatidylcholine, dioleoylphosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine, and the corresponding synthetic phosphatidylethanolamines and phosphatidylglycerols. Cholesterol or other sterols, cholesterol hemisuccinate, glycolipids, cerebrosides, fatty acids, gangliosides, sphingolipids, 1,2-bis(oleoyloxy)-3-(trimethyl ammonio)propane (DOTAP), N-[1-(2,3-dioleoyl) propyl]-N,N,N-trimethylammonium chloride (DOTMA), and other cationic lipids may be incorporated into the liposomes, as is known to those skilled in the art. The relative amounts of phospholipid and additives used in the liposomes may be varied if desired. The preferred ranges are from about 60 to 90 mole percent of the phospholipid; cholesterol, cholesterol hemisuccinate, fatty acids or cationic lipids may be used in amounts ranging from 0 to 50 mole percent. The amounts of antiviral phosphonoacids incorporated into the lipid layer of liposomes can be varied with the concentration of their lipids ranging from about 0.01 to about 50 mole percent.

Using conventional methods, approximately 20 to 30% of the free phosphonoacid present in solution can be entrapped in

liposomes; thus, approximately 70 to 80% of the active compound is wasted. In contrast, where the lipid phosphonoacid is incorporated into liposomes, virtually all of the antiviral compound is incorporated into the liposome, and essentially none of the active compound is wasted.

5 The liposomes with the above formulations may be made still more specific for their intended targets with the incorporation of monoclonal antibodies or other ligands specific for a target. For example, monoclonal antibodies to ... (a) ... may be incorporated into the liposome by

The present invention is described below in detail using the following examples, but the methods disclosed are applicable for the preparation of all phosphonoacids covered by the scope of the invention and are not limited to the examples.

EXAMPLE 1

Synthesis of 1-O-alkyl-2-halo-1,3-propanediol-3-phosphonoformate analogs and 1-O-alkyl-2-amino-1,3-propanediol-3-phosphonoformate analogs

The stereo-controlled synthesis of analogs 1-O-alkyl-2-halo-1,3-propanediol-3-phosphonoformate is outlined in Scheme 1.

1. 2,3-Isopropylidene-*sn*-glycerol, upon treatment with the appropriate alkylmethane sulfonate, leads to the intermediate
2. Removal of the isopropylidene group by treatment with acetic acid followed by tritylation with trityl chloride and pyridine results in compound 3, with a free 2-hydroxyl group. Treatment of 3 with n-halosuccinimide and triphenyl phosphine according to the procedure of Bose and Lal [(1973, Tetrahedron Lett. 40:3937) will lead to intermediate 4. The replacement of the hydroxyl group with halogen proceeds with complete inversion (SN_2 displacement) and in yields from 65-95%. Removal of the trityl group with trifluoroacetic acid in dichloromethane leads to the halo compound 5. Reaction of 5 with carbethoxyphosphodichloridate results in the phosphonoformic acid analog 6.

While this procedure works when X is Cl, Br and I, a slightly different approach is needed for the analog when X=F. Treatment of the intermediate 3 with diphenyltrifluorophosphorane according to a procedure reported by Kobayashi and co-workers (1968, Chem. Pharm. Bull. 16(9):1784) leads to the conversion of alcohol 3 to the fluorinated compound 4 in good yields. Subsequent steps to the fluoro analogs of 6 are identical to those described earlier.

Treatment of bromo intermediate 4 described in Scheme I with liquid ammonia in a steel bomb will result in amination at the 2-position. Treatment of the resulting 2-amino compound with benzyl bromide will protect the 2-amino group.

Subsequent steps will be identical to those described in Scheme I up to intermediate 6. The benzyl protecting group can be removed at this point by hydrogenolysis with Pd/C.

Treatment of the amino intermediate with acyl chloride will result in the N-acyl compound. Alternatively, treatment of the bromo intermediate 4 with mono or dialkyl amine will result in the monoalkyl amino and dialkylamino derivatives.

The procedures described above can be carried out with readily available starting materials, the methodology is well documented and those skilled in the art can recognize the modifications needed in starting materials and methods to synthesize the 2-amino analogs that have been listed.

EXAMPLE 2

Synthesis of thiophosphonoacids and their lipid prodrugs

Thiophosphonoformic acid is synthesized according to the procedure of McKenna (U.S. Patent 5,072,032) by the reaction of trimethyl phosphonoformic acid with Lawesson's reagent [2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane 2,4-disulfide]. Thiophosphonoacetic acid is also synthesized in a similar manner.

Selenophosphonoformic acid can be synthesized by treatment of trimethylphosphonoformic acid with elemental selenium by adaptation of procedures reported by Stec and co-workers (1976, Stec, W.J., Okruszek, A and Michalski, J.J. Org. Chem. 41, 233), and by Buina et al (1979, Buina, N.A.; Sibgatullina, F.G.; Neureldinor, I.A. Izv. Akad. Nauksssr., Ser. Khim. 10, 2362). Selenophosphonoacetic acid can also be synthesized in a similar manner. The selenoacids can then be converted to the corresponding lipid prodrugs by procedures identical to those for the oxo- and thio- analogues.

The synthesis of batyl-thiophosphonoformic acid (PFSA) was carried out in a manner similar to the synthesis of batyl-phosphonoformic acid, with some modifications. 1-O-Octadecylphosphonoformic acid, with some modifications. 1-O-Octadecyl-2-O-benzyl-sn-glycerol, purchased from Bachem (Basel, Switzerland), was coupled to thiophosphonoformic acid ethyl ester using dicyclohexylcarbodiimide. After purification by silica gel chromatography, the PFSA-ethyl ester was

debenzylated with Pd/C and cyclohexene. This procedure, reported in (1977, J. Chem. Soc., Perkin Trans. 1, 490) can be used for hydrogenolysis of compounds containing sulfur. The PFSA was obtained by base hydrolysis of the debenzylated ester. Batyl-thiophosphonoacetic acid was synthesized in a similar manner.

EXAMPLE 3

Synthesis of 1-O-octadecyl-1,3-propanediol-3-phosphonoformate and 1-O-octadecyl-1,2-ethanediol-2-phosphonoformate:

Preparation of 1-O-Octadecyl-1,3-propanediol

1-O-trityl-1,3-propanediol, synthesized according to the procedure of Sela, et al. (1987) Nucleic Acids Research 15:3124, was treated with octadecylmethanesulfonate (NuChek Prep, Inc.) in the presence of sodium hydride in dimethylformamide. The product, 1-O-octadecyl-3-O-tritylpropanediol, was isolated and purified by flash chromatography. The trityl protecting group was removed by treatment with trifluoroacetic acid in dichloromethane to yield 1-O-octadecyl-1,3-propanediol.

To a solution of carbethoxyphosphodichloridate (1.6 mmol) in chloroform (25 ml), cooled to 0°C in an ice salt bath, was added a solution of 1-O-octadecylpropanediol (1 mmol) in pyridine (15 ml), dropwise with stirring. The mixture was allowed to warm to room temperature and stirred at room temperature overnight. The mixture was cooled, and 1 ml water was added. After stirring at 0°C for 2h, the mixture was concentrated in vacuo. The residual oil was flash chromatographed with chloroform-methanol 95:5 as eluent to yield product: 1-O-octadecylpropanediol-3-ethylphosphonoformate.

The ethyl ester was dissolved in 1:1 mixture of ethanol and 0.1N NaOH (50 ml) and sonicated for 15 minutes. The resulting mixture was stirred at 60°C in an oil bath for 2h. The mixture was filtered and the filtrate was concentrated to dryness in vacuo. The resulting solid was resuspended in water, cooled and lyophilized to yield target compound.

The corresponding 1,2-ethanediol analogue may be prepared as described in the above method by substituting 1-O-trityl-1,2-ethanediol as the starting material.

EXAMPLE 4

5 Synthesis of 1-O-octadecyl-1,3-propanediol-3-phosphonoacetate and 1-O-octadecyl-1,2-ethanediol-2-phosphonoacetate:

To a mixture of 1-O-octadecyl-1,3-propanediol (1 mmol) and phosphonoacetic acid ethyl ester (1.1 mmol) in pyridine, 10 (50 ml) cooled to 0°C in an ice bath, was added a solution of dicyclohexylcarbodiimide (DCC; 3 mmol) in CH₂Cl₂ (20 ml) dropwise with stirring. The mixture was stirred at 0°C for 2h and at room temperature overnight. The mixture was concentrated to dryness and the residue was flash 15 chromatographed with chloroform-methanol 95:5 as eluent to yield 1-O-octadecylpropanediol-3-phosphonoacetate ethyl ester.

The ethyl ester was dissolved in a 1:1 mixture of 0.1N NaOH and ethanol (50 ml). The mixture was sonicated for 15 minutes and heated in an oil bath at 60°C for 2h. The mixture 20 was filtered, and the filtrate was concentrated in vacuo. The resultant residue was dissolved in water, cooled and lyophilized to yield product as a fluffy white solid.

The corresponding 1,2-ethanediol analogue may be prepared as described in the above method by substituting 1-O-trityl- 25 1,2-ethanediol as the starting material.

EXAMPLE 5

30 Synthesis of 1-O-octadecyl-1,3-propanediol-3-thiophosphonoformate:

To a mixture of 1-O-octadecyl-1,3-propanediol (1 mmol) and ethythiophosphonoformate (1.1 mmol) in pyridine (50 ml) cooled to 0°C in an ice-salt bath was added a solution of dicyclohexylcarbodiimide (DCC; 3 mmol) in dichloromethane 35 dropwise with stirring. The resulting mixture was stirred at 0°C for 2h and at room temperature overnight. The mixture was filtered, and concentrated to dryness in vacuo. The residue was flash chromatographed over silica gel with an increasing

gradient of methanol in chloroform as eluent to yield pure product.

The product was dissolved in a mixture of ethanol:0.1N NaOH (1:1, 50 ml) and sonicated for 15 minutes. The resulting mixture was heated at 60°C for 2h, filtered and concentrated to dryness. The residue was dissolved in a minimum amount of water and lyophilized to yield the target compound as a fluffy solid.

EXAMPLE 6

Synthesis of 1-O-octadecyl-1,3-propanediol-3-thiophosphonoacetate:

To a mixture of 1-O-octadecyl-1,3-propanediol (1 mmol) and ethyl thiophosphonoacetate (1.1 mmol) in CH₂Cl₂ (50 ml) cooled to 0°C in an ice salt bath, was added a solution of dicyclohexylcarbodiimide (3.3 mol), dropwise, with stirring. The resulting mixture was stirred at room temperature overnight. The mixture was concentrated to dryness and flash chromatographed over silica gel with chloroform-methanol (95:5) as eluent to yield pure product.

The ethyl ester was dissolved in a 1:1 mixture of ethanol and 0.1N NaOH (50 ml) and sonicated for 15 minutes. The resulting mixture was heated at 60°C for 2h, filtered and concentrated to dryness. The residue was dissolved in minimum amount of water and lyophilized to yield pure compound as a fluffy solid.

EXAMPLE 7

Synthesis of 1-O-octadecyl-2-O-methyl-sn-glycero-3-phosphonoformate:

To a solution of carbethoxyphosphodichloridate (1.6 mmol) in chloroform (20 ml), cooled to 0°C in an ice salt bath was added a solution of 1-O-octadecyl-2-O-methyl-sn-glycerol (1.0 mmol) in pyridine (15 ml) dropwise. The mixture was stirred at room temperature overnight. The mixture was cooled, 2 ml of water added and the resulting mixture stirred at room temperature for 2h. The reaction mixture was then

concentrated in vacuo, and the residue was flash chromatographed over silica gel with chloroform:methanol as eluent.

The ethyl ester was dissolved in a 1:1 mixture of ethanol and 0.1N NaOH and sonicated for 15 minutes. The mixture was heated with stirring at 60°C for 2h, filtered and the filtrate evaporated to dryness. The residue was recrystallized from 25% aqueous ethanol to yield pure product.

EXAMPLE 8

10 Synthesis of 1-O-octadecyl-2-O-methyl-sn-glycero-3-phosphonoacetate:

To a mixture of 1-O-octadecyl-2-O-methyl-sn-glycerol (1 mmol) and phosphonoacetic acid ethyl ester hydrochloride (1 mmol) in pyridine (50 ml), cooled to 0°C in an ice salt bath was added a solution of dicyclohexylcarbodiimide (3.0 mmol) in CH₂Cl₂ (20 ml), dropwise with stirring. The resulting solution was stirred at room temperature overnight. After concentrating in vacuo, the residue was flash chromatographed over silica gel with a gradient of methanol in chloroform as eluent to yield 1-O-octadecyl-2-O-methyl-sn-glycero-3-phosphonoacetic acid ethyl ester.

The ethyl ester was dissolved in a 1:1 mixture of ethanol: 0.1N NaOH (50 ml), and the resulting solution was sonicated for 15 minutes. The solution was then heated in an oil bath at 70°C for 3h. The solution was cooled, the resulting solid was filtered off and washed with cold ethanol. The solid was vacuum dried to yield pure product.

EXAMPLE 9

30 Synthesis of 1-O-octadecyl-2-O-methyl-sn-glycero-3-thiophosphonoformate:

To a mixture of 1-O-octadecyl-2-O-methyl-sn-glycerol (1 mmol) and ethyl thiophosphonoformate (1.1 mmol) in dichloromethane (50 ml), cooled to 0°C in an ice salt bath was added a solution of dicyclohexylcarbodiimide (3 mmol) in CH₂Cl₂ (20 ml), dropwise, with stirring. The resulting mixture was stirred at room temperature overnight. The reaction mixture

was filtered and concentrated to dryness in vacuo. The residue was flash chromatographed over silica gel with an increasing gradient of methanol in chloroform as the eluting solvent to yield pure 1-O-octadecyl-2-O-methyl-sn-glycero-3-thiophosphonoformic acid ethyl ester.

The ethyl ester was dissolved in a 1:1 mixture of ethanol and 0.1N NaOH (50 ml) and sonicated for 15 minutes. The solution was heated for 2h at 60°C, filtered, and the filtrate concentrated to dryness. The residue was dissolved in minimum amount of ester and lyophilized to yield the target compound as a fluffy solid.

EXAMPLE 10

Synthesis of 1-O-octadecyl-2-O-methyl-sn-glycero-3-thiophosphonoacetate:

To a mixture of 1-O-octadecyl-2-O-methyl-sn-glycerol (1 mmol) and ethyl thiophosphonoacetate (1 mmol) in pyridine (50 ml), was added a solution of dicyclohexylcarbodiimide (DCC; 3.0 mmol) dropwise with stirring. The mixture was stirred at room temperature overnight. Solvent was removed in vacuo, and the residue was flash chromatographed over silica gel with an increasing gradient of methanol in chloroform as eluent to yield pure 1-O-octadecyl-2-O-methyl-sn-glycero-3-thiophosphonoacetic acid ethyl ester.

The ethyl ester was dissolved in a 1:1 mixture of ethanol and 0.1N NaOH (50 ml). The solution was sonicated for 15 minutes and stirred at 65°C for 2h. The solution was filtered and the filtrate cooled in the freezer. The resulting solid was filtered off, washed with cold ethanol and dried in vacuo to give pure product.

EXAMPLE 11

Synthesis of 1-O-octadecyl-2-O-benzyl-sn-glycero-3-thiophosphonoformic acid ethyl ester:

To a solution of 1-O-octadecyl-2-O-benzyl-sn-glycerol (1 mmol) and ethyl thiophosphonoformate (1 mmol) in pyridine (50 ml), cooled to 0°C in an ice-salt bath, was added a solution

of dicyclohexylcarbodiimide (3 mmol) in dichloromethane (20 ml) dropwise with stirring. The mixture was stirred at room temperature overnight. After concentration in vacuo, the residue was chromatographed over silica gel to yield pure target compound.

EXAMPLE 12

Synthesis of 1-O-octadecyl-sn-glycero-3-thiophosphonoformate:

1-O-octadecyl-2-O-benzyl-sn-glycero-3-thiophosphonoformic acid ethyl ester (1 mmol) was dissolved in ethanol (25 ml). Pd/C (100 mg) and cyclohexene (5 ml) was added to the solution and the mixture stirred at room temperature overnight. The mixture was filtered and the filtrate was concentrated in vacuo. Purification of the resulting residue by flash chromatography with a gradient of methanol in chloroform resulted in the debenzylated ethyl ester. This ester was dissolved in a 1:1 mixture of ethanol and 0.1 NaOH (50 ml) and the solution was sonicated for 15 minutes. After heating at 60°C for 2h, the solution was filtered and the filtrate concentrated in vacuo. The residue was suspended in water, cooled and lyophilized to yield target compound.

EXAMPLE 13

Synthesis of 1-O-octadecyl-2-O-benzyl-sn-glycero-3-thiophosphonoacetic acid ethyl ester:

To a solution of 1-O-octadecyl-2-O-benzyl-sn-glycerol (1 mmol) and ethyl thiophosphonoacetate (1 mmol) in pyridine (50 ml), cooled to 0°C in an ice-salt bath, was added a solution of dicyclohexylcarbodiimide (3 mmol) in dichloromethane (20 ml) dropwise with stirring. The mixture was stirred at room temperature overnight, and concentrated to dryness in vacuo. The residue was flash chromatographed with a gradient of methanol in chloroform as eluent to yield pure target compound.

EXAMPLE 14

Synthesis of 1-O-octadecyl-sn-glycero-3-thiophosphonoacetate:

A solution of 1-O-octadecyl-2-O-benzyl-sn-glycero-3-phosphonoacetic acid ethyl ester (1 mmol) in ethanol (50 ml) was combined with Pd/C (100 mg) and cyclohexene (5 ml) and the mixture was hydrogenated at 60 psi hydrogen overnight.

5 The mixture was filtered, and the filtrate concentrated in vacuo. The residue was flash chromatographed with a gradient of methanol in chloroform as eluent to yield the debenzylated ethyl ester. This was dissolved in a 1:1 mixture of ethanol and 0.1N NaOH (50 ml), and sonicated for 15
10 minutes. The mixture was heated at 60° for 2h. The solution was filtered and concentrated in vacuo. The product was recrystallized from aqueous ethanol.

The following examples refer to the intermediate and target compounds numbered as shown in synthesis Schemes II and
15 III.

EXAMPLE 15

Synthesis of phosphonoacid species wherein Y = CH-OH, m = 1, R1 = octadecyl (Compound #8 on Scheme II):

20 D-erythrose, 1, (purchased from Aldrich Chemical Company), upon treatment with NaH and benzyl bromide in DMF at -70°C will give the selectively protected species 4-O-benzyl-erythrose, 2. Treatment of this intermediate with
25 dimethoxypropane and acetone with trace amounts of perchloric acid will lead to 2,3-di-O-isopropylidene-4-O-benzyl erythrose 3. Reduction of compound 3 with sodium borohydride leads to the protected erythritol, 4. Treatment of 4 with octadecylmethanesulfonate yields the 1-O-octadecyl-2,3,di-O-isopropylidene-4-O-benzyl erythritol 5. Debenzylation with
30 Pd/C and hydrogen followed by DCC coupling with ethyl phosphonoformate will yield intermediate 7. Deblocking of 1 with 10% TFA in CH₂Cl₂, followed by base hydrolysis yields target compound 8.

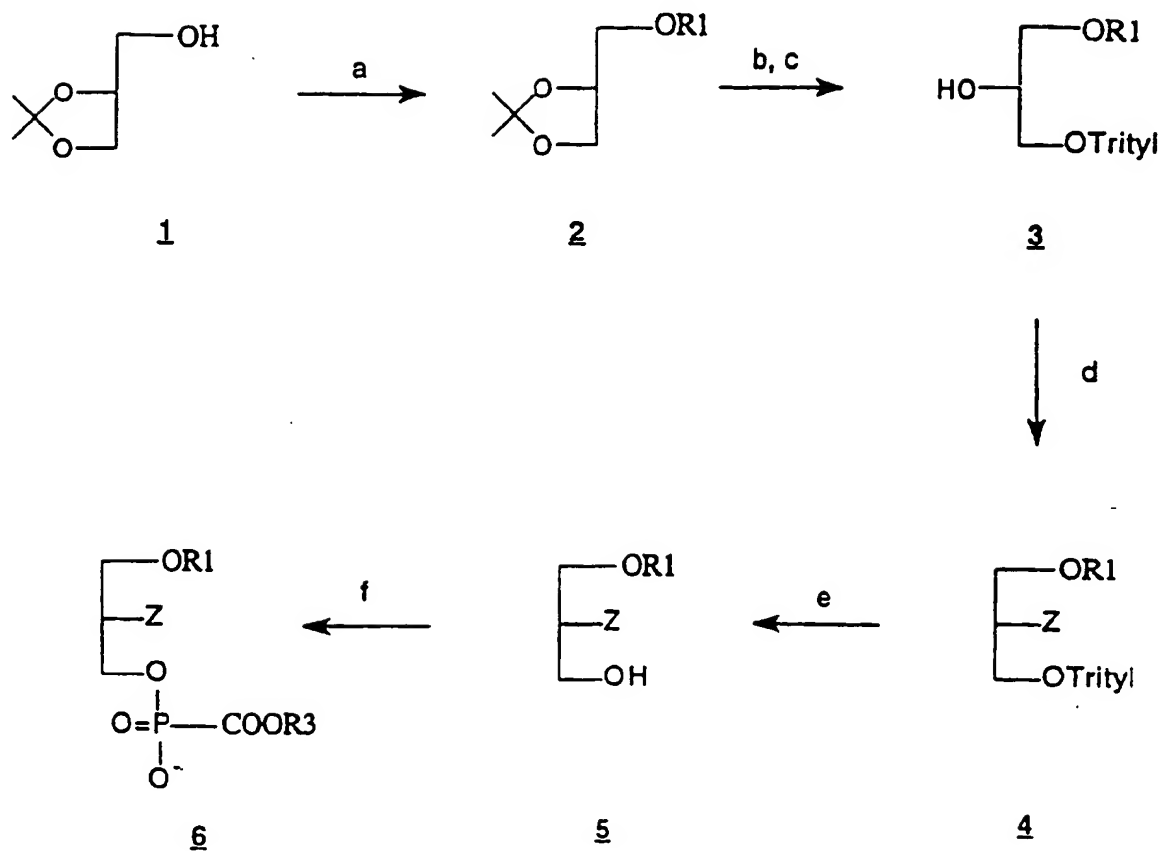
EXAMPLE 16

Synthesis of Phosphonoformate derivative wherein
 $R_1 = H$, $Y = CH-OH$, $R = \text{octadecyl}$, $m = 2$ (compound 17 on Scheme
III).

5 Commercially available D-ribose, 9, (Sigma Chemical Co)
upon treatment with trimethylsilyl methylmercaptan by
adaptation of a procedure by Evans and Co-workers (Evans,
D.A., Truesdale, L.K., Gimm, K.G., Nesbitt, S.L., J. Am. Chem.
10 Soc., 99, 5009, 1977) will result in the dimethyldithioacetal
10. This locks the ribose in the open chain conformation.
Treatment of the protected ribose with benzyl bromide in DMF
at -70° will result in selective blocking of the 5-primary
hydroxyl group of ribose leading to compound 11. Such
15 selective blocking has been reported in the literature (1987,
Fukuzawa, A., Sato, H., Masamune, T. Tetrahedron Lett. 28,
4303). The 2,3, and 4 hydroxyl groups on the 1,5 derivatized
ribose can be protected by treatment with methoxymethyl
chloride (1972, Stark, G., Takashi, T. J. Am. Chem. Soc., 94,
20 7827) to yield the fully protected ribose intermediate 12.

The aldehyde at the C_1 position is regenerated by
treatment of intermediate 12 with $AgNO_3/Ag_2O$ (1977, Corey,
E.J., Shibasaki, M., Knolle, J., Sugahara, T. Tetrahedron
Lett., 785) to yield compound 13. Reduction with sodium
25 borohydride followed by alkylation with
octadecylmethanesulfonate yields the 1-O-octadecyl-2,3,4-tri-
O-methoxymethyl-5-O-benzyl ribose 15.

Removal of the benzyl group by hydrogenation with Pd/C
followed by coupling with ethyl phosphonoformate using
30 dicyclohexyl carbodiimide yields the intermediate 16.
Treatment with acetic acid removes the methoxymethyl
protecting groups, and treatment with base leads to the target
compound, 17.

SCHEME 1

$R_1 = -(\text{CH}_2)_n\text{CH}_3$, $n = 7, 9, 11, 13, 15, 17$

$Z = \text{Cl, Br, I, } -\text{OCH}_3, -\text{NH}_2, -\text{NH-R}_2, -\text{N}-(\text{R}_2)_2$

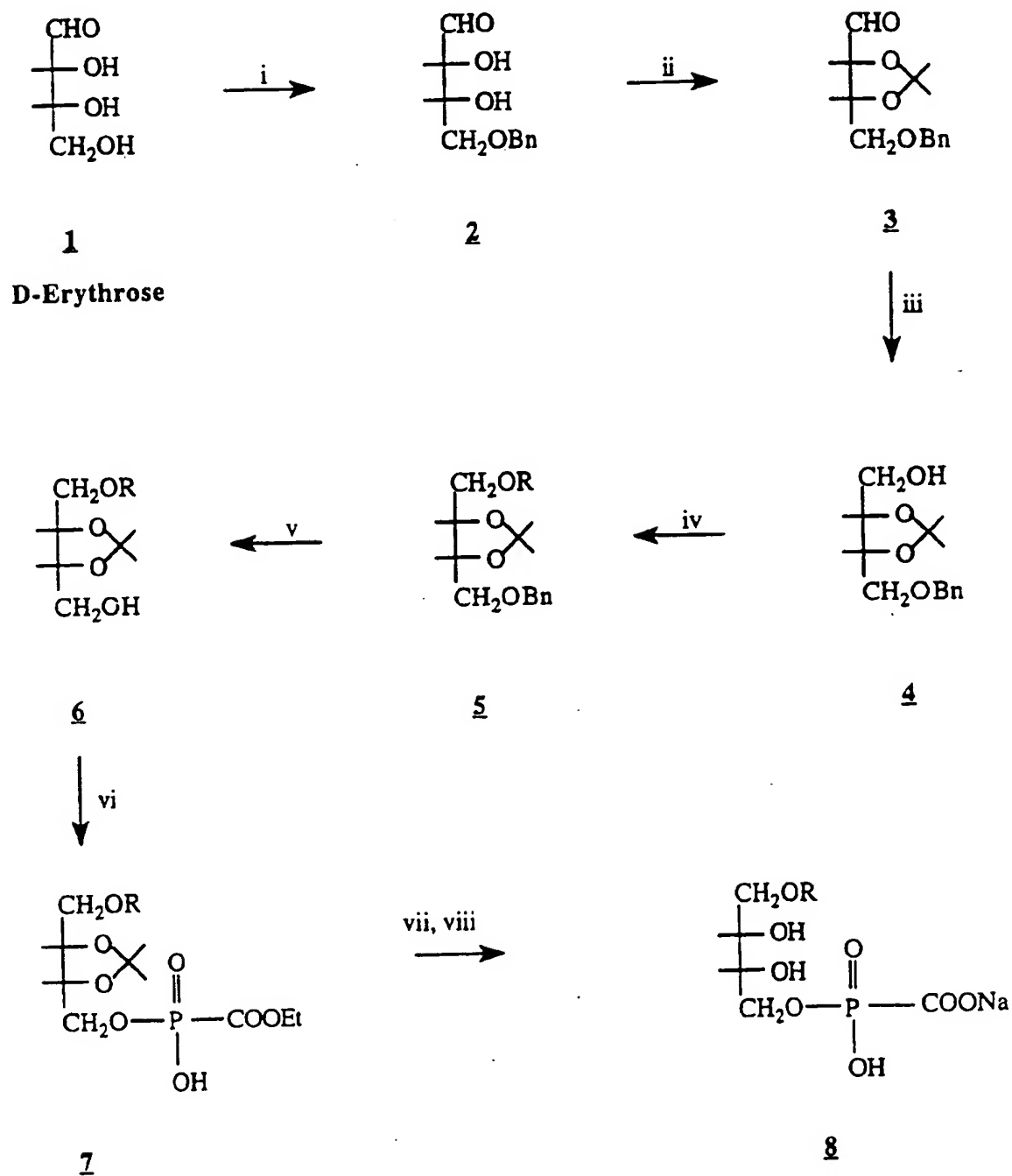
$R_3 = -\text{CH}_3, -\text{CH}_2\text{CH}_3 \text{ and } -\text{CH}_2\text{CH}_2\text{CH}_3$

a: NaH, DMF, $R_1\text{-OSO}_2\text{Me}$; b: Acetic acid; c: Trityl chloride, pyridine;

d: NaH, DMF, $R_2\text{-Br}$; e: TFA, CH_2Cl_2 ; f: $\text{Cl}_2\text{POCOOR}_3$; H_2O

SCHEME II

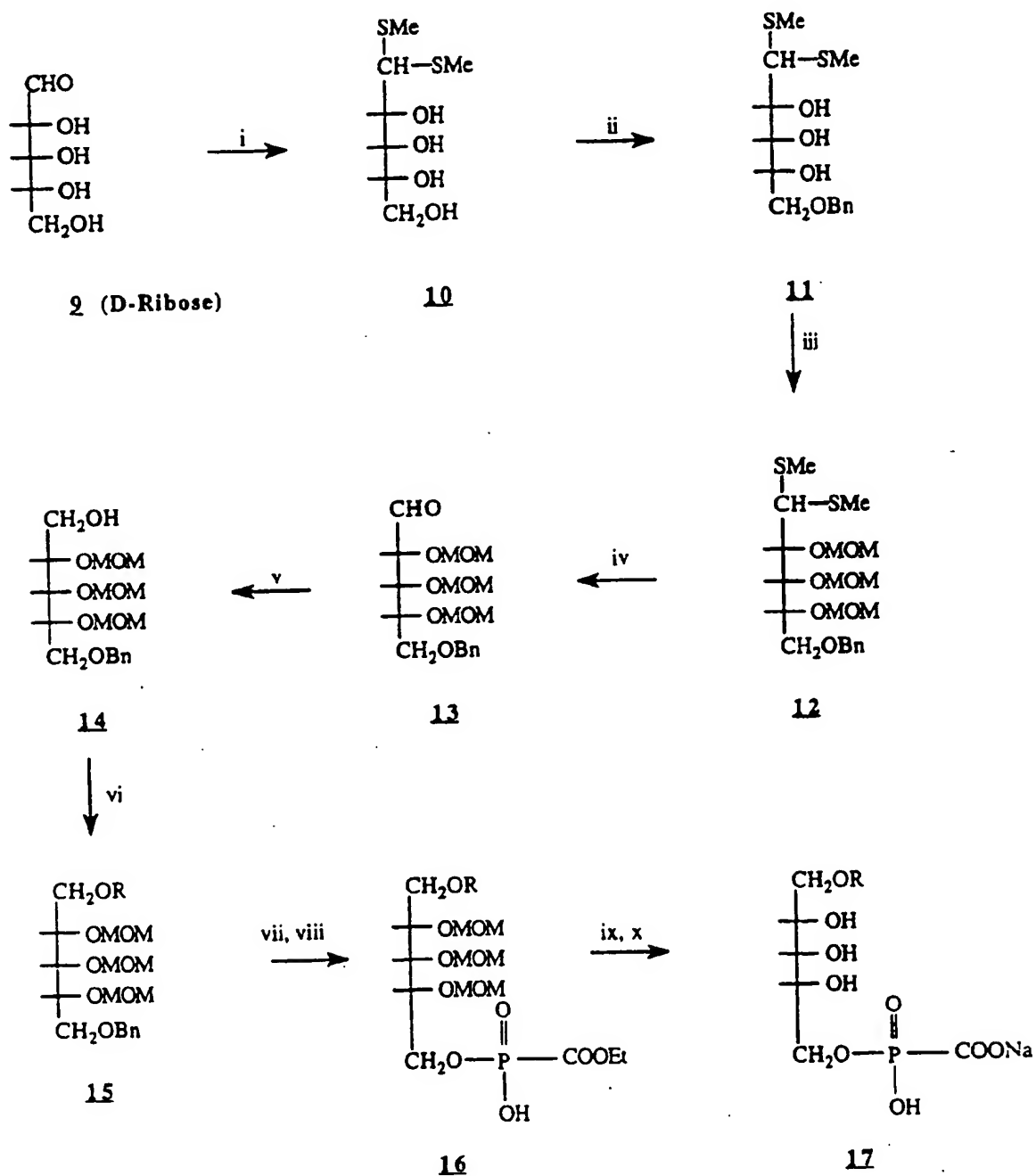
Example: Synthesis of phosphonoformic acid analog $Y=CHOH$, $m=1$, $R_2=OH$,
 $R_1=Octadecyl$



- i. $BnBr$, NaH ; ii. DMP , H^+ ; iii. $NaBH_4$; iv. $ROSO_2Me$ ($R=Octadecyl$), NaH ;
 v. H_2 , Pd/C ; vi. DCC , ethyl phosphonoformate; vii. TFA , CH_2Cl_2 ;

SCHEME III

Example: Synthesis of phosphonoformic acid analog $Y=CHOH$, $m=2$, $R_2=OH$,
 $R_1=Octadecyl$



i. MeSSiMe₃, ZnI₂; ii. BnBr, NaH; iii. CH₃OCH₂Cl (MOM chloride), NaH, THF;
 iv. AgNO₃, Ag₂O; v. NaBH₄; vi. ROSO₂Me, NaH; vii. H₂, Pd/C; viii. DCC, ethyl
 phosphonoformate; ix. Acetic acid; x. NaOH, ethanol

EXAMPLE 17

Antiviral activity of phosphonoacid prodrugs in cell culture

Phosphonoacid derivatives having structures according to formula I were tested for antiviral activity in human embryonic lung fibroblasts (MRC-5) or in human epithelioid cervical carcinoma cells (HeLa) expressing the CD4 receptor (HT4-6C). HCMV (strain AD-169) and HSV-1 (wild type) were used to infect MRC-5 cells and HCMV or HSV-specific DNA was measured by DNA-probe methods (Hybriwix™, Diagnostic Hybrids, Inc., Athens, OH) according to the manufacturer's instructions. HIV replication was assessed in HIV-1 infected HT4-6C cells using a plaque reduction assay (Larder, B. et al. (1990) Antimicrobial Agents Chemother. 34:436).

The cytotoxicity of B-PFA and B-PAA in subconfluent MRC-5 cells was assessed by trypan blue exclusion. The toxic dose, TD_{50} was $>1000\mu M$ and for B-PAA was 32-100 μM , indicating that the compounds have selectivity indexes which are 300 or greater.

EXAMPLE 18

CMV antiviral susceptibility assay

Subconfluent MRC-5 cells in 24-well culture dishes were pretreated for 24 hours with various concentrations of drug in MEM medium containing 2% FBS and antibiotics. The media was removed and virus added at a dilution that will result in a 3-4+ cytopathic effect (CPE) in the no-drug wells in five days. This was absorbed for 1 hour at 37°C, aspirated and replaced with the drug dilutions. After five days of incubation HCMV DNA was quantified in triplicate by nucleic acid hybridization using a CMV Antiviral Susceptibility Test Kit from Diagnostic Hybrids, Inc. (Athens, OH). The media was removed and cells lysed according to the manufacturer's instructions. After absorption of the lysate, the Hybriwix™ filters were hybridized overnight at 60°C. The Hybriwix™ were washed for 30 minutes at 73°C and counted in a gamma counter. The results are expressed as a percentage of the untreated HCMV-infected control cells.

EXAMPLE 19

HSV antiviral susceptibility assay

Subconfluent MRC-5 cells in 24-well culture dishes were inoculated by removing the media and adding HSV-1 virus at a dilution that will result in a 3-4+ CPE in the no-drug well in 20-24 hours. This was absorbed for 1 hour at 37°C, aspirated and replaced with various concentrations of drugs in MEM medium containing 2% FBS and antibiotics. After approximately 24 hours of incubation, HSV DNA was quantified in triplicate by nucleic acid hybridization using a HSV Antiviral Susceptibility Test Kit from Diagnostic Hybrids, Inc. (Athens, OH). The media was removed and cells lysed according to the manufacturer's instructions. After absorption of the lysate, the Hybriwix™ filters were hybridized overnight at 60°C. The Hybriwix™ were washed for 30 minutes at 73°C and counted in a gamma counter. The results are expressed as a percentage of the untreated HSV-infected control cells.

EXAMPLE 20

HT4-6C Cells

HT4-6C cells and plaque reduction assay, CD4-expressing HeLa cells, HT4-6C cells (Chesebro, B. and K. Wehrly (1988) J. Virology 62:3779-3788), were obtained from Bruce Chesebro, Hamilton, Mont. The effect of antiviral compounds on HIV replication was measured by a plaque reduction assay. Briefly, monolayers of HT4-6C cells were infected with 100 to 300 PFU of virus per well in 24-well microdilution plates. Various concentrations of drug were added to the culture medium, Dulbecco's Modified Eagle Medium containing 5% fetal bovine serum and antibiotics, as noted above. After 3 days at 37°C, the monolayers were fixed with 10% formaldehyde solution in phosphate-buffered saline and stained with 0.25% crystal violet to visualize virus plaques (Larder, B. et al. (1989) Science 243:1731-1734). Antiviral activity was assessed as the percentage of control plaques measured in drug-treated samples.

TABLE I. ANTI-HUMAN CYTOMEGALOVIRUS ACTIVITY OF PHOSPHONOFORMATE ACID AND VARIOUS PRODRUGS

	Compound	IC ₅₀ , μ M	-fold increase in activity	p value
5	PFA	46 \pm 19 (4)	-	-
	B-PFA	0.43 \pm 0.27 (9)	107	<0.0001
10	3B-PFA	0.68;0.72 (2)	68	n.d.
	(rac)B-PFA	0.60;0.78 (2)	77	n.d.
	B-PFA-OEt	1.2 \pm 0.48 (4)	38	0.0033
	B-PFA-OMe	1.08 \pm 0.36 (3)	43	0.0104
15	BB-PFA-OEt	2.9 \pm 0.97 (4)	16	0.0040
	BB-PFA	0.64 \pm 0.65 (8)	72	<0.0001
	MB-PFA	0.64 \pm 0.14 (3)	72	0.0050
20	DMG-PFA	125 \pm 43 (5)	0.3	0.0117
	DMG-PFA-OEt	30 \pm 18 (4)	1.5	0.2673
	HDP-PFA	0.91 \pm 0.60 (3)	51	0.0102
	ODP-PFA	0.90 (1)	51	n.d.
25	OLP-PFA	0.37 (1)	124	n.d.
	DDP-PFA	0.60;0.94 (2)	60	n.d.

Data are Mean \pm S.D. Number in parentheses (n) is the number of replicates. p values versus PFA were determined by Student's T test of unpaired means using Instat2 Statistical Software (Graph Pad Software, San Diego, CA). n.d. - not determined.

ABBREVIATIONS: PFA, phosphonoformate; B-PFA, 1-O-octadecyl-*sn*-glycero-3-phosphonoformate; BB-PFA, 1-O-octadecyl-2-O-benzyl-*sn*-glycero-3-phosphonoformate; B-PFA-OEt, 1-O-octadecyl-*sn*-glycero-3-phosphonoformate ethyl ester; B-PF-OMe, 1-O-octadecyl-*sn*-glycero-3-phosphonoformate methyl ester; BB-PFA-OEt, 1-O-octadecyl-2-O-benzyl-*sn*-glycero-3-phosphonoformate ethyl ester; MB-PFA, 1-O-octadecyl-2-O-methyl-*sn*-glycero-3-phosphonoformate; DMG-PFA, 1,2-dimyristoyl-*sn*-glycero-3-phosphonoformate; DMG-PFA-OEt, 1,2-dimyristoyl-*sn*-glycero-3-phosphonoformate ethyl ester; 3B-PFA, 3-O-octadecyl-*sn*-glycero-1-PFA; (rac)B-PFA; 1-O-octadecyl-*rac*-glycero-3-PFA; HDP-PFA, 1-O-hexadecylpropanediol-3-PFA; ODP-PFA, 1-O-octadecylpropanediol-3-PFA; OLP-PFA, 1-O-oleylpropanediol-3-PFA; DDP-PFA, 1-O-dodecylpropanediol-3-PFA. IC₅₀ - μ M concentration of drug which inhibits viral replication by 50%.

TABLE II. ANTI-HUMAN CYTOMEGALOVIRUS ACTIVITY OF PHOSPHONOACETATE AND PHOSPHONOACETATE PRODRUGS

	Compound	IC ₅₀ , μ M (n)	-fold increase in activity	p value
5	PAA	22 \pm 7 (5)	-	-
	B-PAA	0.22 \pm 0.08 (8)	100	<0.0001
	BB-PAA	0.92 \pm 0.80 (7)	24	<0.0001
10	B-PAA-OEt	3.6 \pm 1.5 (7)	6.1	<0.0001
	BB-PAA-OEt	7.2 \pm 1.0 (3)	3.1	<0.0062
	BB-(C)-PAA	1.4 \pm 0.9 (3)	16	0.0013
	MB-PAA	0.61 \pm 0.76 (6)	36	<0.0001
15	MB-PAA-OEt	6.7 \pm 1.9 (3)	3.3	0.0057
	DMG-PAA	19 \pm 6.5 (4)	1.2	0.2651
	DMG-PAA-OEt	108 \pm 42 (3)	0.2	0.0016
20	DMP-PAA	93 \pm 92 (3)	0.2	0.0593
	DMP-PAA-OEt	130 \pm 28 (2)	0.2	0.0001
	DPP-PAA	80 \pm 42 (2)	0.3	0.0086

25 ABBREVIATIONS: PAA, phosphonoacetate; B-PAA, 1-O-octadecyl-sn-glycero-3-phosphonoacetate; BB-PAA, 1-O-octadecyl-2-O-benzyl-sn-glycero-3-phosphonoacetate; B-PAA-OEt, 1-O-octadecyl-sn-glycero-3-phosphonoacetate ethyl ester; BB-PAA-OEt, 1-O-octadecyl-2-O-benzyl-sn-glycero-3-phosphonoacetate ethyl ester; BB-(C)-PAA, 1-O-octadecyl-2-O-benzyl-sn-glycero-3-carboxymethylenephosphonate; MB-PAA, 1-O-octadecyl-2-O-methyl-sn-glycero-3-phosphonoacetate; DMG-PAA, 1,2-dimyristoyl-sn-glycero-3-phosphonoacetate; DMG-PAA-OEt, 1,2-dimyristoyl-sn-glycero-3-phosphonoacetate ethyl ester; DMP-PAA, 1,2-dimyristoyl-sn-glycero-3-phospho-3-phosphonoacetate; DMP-PAA-OEt, 1,2-dimyristoyl-sn-glycero-3-phospho-phosphonoacetate ethyl ester; DPP-PAA, 1,2-dipalmitoyl-sn-glycero-3-phospho-phosphonoacetate.

TABLE III. INHIBITION OF HUMAN HERPES SIMPLEX VIRUS 1
REPLICATION BY PHOSPHONOFORMATE AND
PHOSPHONOFORMATE PRODRUGS

Compound	IC ₅₀ , μ M (n)	-fold increase in activity	p value
PFA	47 \pm 20 (6)	-	-
B-PFA	1.1 \pm 1.1 (5)	43	0.0003
BB-PFA	1.4 \pm 0.4 (4)	34	0.0003
MB-PFA	0.65 \pm 0.21 (4)	72	0.0009

Data are Mean \pm S.D. Number in parentheses (n) is the number of replicates. P values versus PFA were determined by student's T test using InStat2 Statistical Software (Graph Pad Software, San Diego, CA).

ABBREVIATIONS: PFA, phosphonoformate; B-PFA, 1-O-octadecyl-sn-glycero-3-phosphonoformate; BB-PFA, 1-O-octadecyl-2-O-benzyl-sn-glycero-3-phosphonoformate; MB-PFA, 1-O-octadecyl-2-O-methyl-sn-glycero-3-phosphonoformate.

TABLE IV. ANTIVIRAL EFFECT OF PHOSPHONOFORMATE AND PHOSPHONOFORMATE PRODRUGS ON HUMAN IMMUNODEFICIENCY VIRUS-1 REPLICATION IN HT4-6C CELLS

	Compound	IC ₅₀ μ M			-fold increase in activity	p value
5	PFA	133	\pm 54	(8)	-	-
10	B-PFA	3.60 \pm 1.51		(3)	37	0.0015
	3B-PFA	0.9		(1)	222	n.d.
	(rac)B-PFA	1.3		(1)	102	n.d.
	BB-PFA	14.8 \pm 7.3		(2)	9	0.0091
15	MB-PFA	0.8 \pm 0.7		(4)	166	0.0014†
	thio-ODG-PFA	2.4;2.3		(2)	57	n.d.
	thio-HDG-PFA	2.8		(1)	48	n.d.

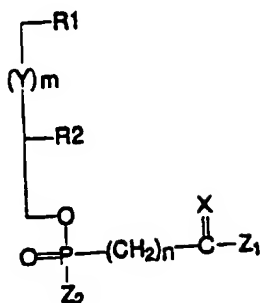
20 Data are Mean \pm S.D. Number in parentheses (n) is the number of replicates. p values versus PFA were determined by Student's T test of unpaired means using Instat2 Statistical Software (Graph Pad Software, San Diego, CA). n.d. - not determined. †p=0.0373 versus B-PFA.

25 ABBREVIATIONS: PFA, phosphonoformate; B-PFA, 1-O-octadecyl-sn-glycero-3-phosphonoformate; BB-PFA, 1-O-octadecyl-2-O-benzyl-sn-glycero-3-phosphonoformate; MB-PFA, 1-O-octadecyl-2-O-methyl-sn-glycero-3-phosphonoformate; 3B-PFA, 3-O-octadecyl-sn-glycero-1-PFA; (rac)B-PFA; 1-O-octadecyl-rac-glycero-3-PFA; HDP-PFA, 1-O-hexadecylpropanediol-3-PFA; thio-ODG-PFA, 1-S-octadecyl-sn-glycero-3-PFA; thio-HDG-PFA, 1-S-hexadecyl-sn-glycero-3-PFA. IC₅₀ - μ M concentration of drug which inhibits viral replication by 50%.

35 The invention may be embodied in other specific forms without departing from its spirit of essential characteristics. The described embodiments are to be considered in all respects only as illustrative and not restrictive, and the scope of the invention is therefore indicated by the appended claims rather than by the foregoing description. All
40 modifications which come within the meaning and range of the lawful equivalency of the claims are to be embraced within their scope.

WHAT IS CLAIMED IS

1. A compound of the formula



wherein

R1 is an O-alkyl or S-alkyl group, wherein the alkyl group comprises a linear or branched, substituted or unsubstituted C_1 to C_{24} group, having from 0 to 6 double bonds; or

R1 is an O-acyl or S-acyl moiety, wherein the acyl group comprises a linear or branched, substituted or unsubstituted C_1 to C_{24} group, having from 0 to 6 double bonds;

Y is HC-R2;

m = 0 or a whole number from 1 to 6;

each R2 is independently selected from the group consisting of R1; linear or branched, substituted or unsubstituted N-acyl, N-alkyl and N-dialkyl C_1 to C_{24} groups, each acyl or alkyl group having from 0 to 6 double bonds; OH; H; OCH_3 ; O-benzyl; SH; SCH_3 ; NH_2 ; or from the group consisting of fluorine, chlorine, iodine or bromine;

Z_1 and Z_2 are independently R_3 ; or $\text{O}^- \text{A}^+$; wherein

R_3 is selected from the group of alcohols consisting of substituted or unsubstituted linear C_1 to C_6 alkyl groups, substituted or unsubstituted branched C_3 to C_6 alkyl groups, CH_2Ph ; and $\text{CH}_3(\text{CH}_2)_n\text{NH}_2$, wherein n is 0 to 8; or

R_3 is selected from the group consisting of $(\text{CH}_3)_3\text{N}^+\text{CH}_2\text{CH}_2\text{OH}$, $\text{HOCH}_2\text{CH}_2\text{NH}_2$, $\text{HOCH}_2\text{CH}(\text{NH}_2)\text{CO}_2\text{H}$, $\text{C}_6\text{H}_{12}\text{O}_6$, $\text{CH}_2\text{OHCHOHCH}_2\text{OH}$; pentoses, hexoses and their corresponding alcohols;

A' is selected from the group consisting of Na⁺; K⁺; H⁺; NH₄⁺; amines selected from the group consisting of mono-, di-, and trialkylamines, and other physiologically acceptable cations;

X is O, S or Se; and

5 n = is 0 or 1;

provided that when X is O and m=0, R2 is not OH.

2. A compound according to Claim 1 wherein m is 0.

3. A compound according to Claim 1 wherein X is oxygen.

10 4. A compound according to Claim 1 wherein R1 is an O-alkyl group.

5. A compound according to Claim 1 wherein R1 is an O-octadecyl group.

6. A compound according to Claim 1 wherein R1 is an O-oleyl group.

15 7. A compound according to Claim 1 wherein R2 is an O-benzyl group.

8. A compound according to Claim 1 wherein R2 is an OCH₃ group.

9. A compound according to Claim 1 wherein R3 is a methyl group.

10. A 1-O-octadecyl-1,3-propanediol-3-phosphonoacid.

20 11. A 1-O-octadecyl-1,3-propanediol-3-thiophosphonoacid.

12. A 1-O-octadecyl-1,3-propanediol-3-selenophosphonoacid.

13. A 1-O-octadecyl-2-O-methyl-sn-glycero-3-phosphonoacid.

14. A 1-O-octadecyl-2-O-methyl-sn-glycero-3-thiophosphonoacid.

15. A 1-O-octadecyl-2-O-methyl-sn-glycero-3-selenophosphonoacid.

25 16. A 1-O-octadecyl-2-O-benzyl-sn-glycero-3-phosphonoacid.

17. A 1-O-octadecyl-2-O-benzyl-sn-glycero-3-thiophosphonoacid.

18. A 1-O-octadecyl-2-O-benzyl-sn-glycero-3-selenophosphonoacid.

19. A 1-O-octadecyl-sn-glycero-3-phosphonoacid methyl ester.

20. A 1-O-octadecyl-sn-glycero-3-thiophosphonoacid methyl ester.

30 21. A 1-O-octadecyl-sn-glycero-3-selenophosphonoacid methyl ester.

22. A 1,2-dimyristoyl-sn-glycero-3-phosphonoacid ethyl ester.

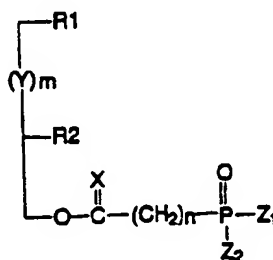
23. A 1,2-dimyristoyl-sn-glycero-3-thiophosphonoacid ethyl ester.

24. A 1-O-oleyl-sn-glycero-3-phosphonoacid.

25. A 1-O-oleyl-1,3-propanediol-3-phosphonoacid.
 26. A 1-O-oleyl-2-O-methyl-*sn*-glycero-3-phosphonoacid.
 27. A 1,2-dimyristoyl-*sn*-glycero-3-selenophosphonoacid ethyl

ester.

28. A compound having the formula



wherein

R1 is an O-alkyl or S-alkyl group, wherein the alkyl group comprises a linear or branched, substituted or unsubstituted C₁ to C₂₄ group, having from 0 to 6 double bonds; or

R1 is an O-acyl or S-acyl moiety, wherein the acyl group comprises a linear or branched, substituted or unsubstituted C₁ to C₂₄ group, having from 0 to 6 double bonds;

Y is HC-R2;

m = 0 or a whole number from 1 to 6;

each R2 is independently selected from the group consisting of R1; linear or branched, substituted or unsubstituted N-acyl or N-alkyl or N-dialkyl C₁ to C₂₄ groups, each acyl or alkyl group having from 0 to 6 double bonds; OH; H; OCH₃; O-benzyl; SH; SCH₃; NH₂; or from the group consisting of fluorine, chlorine, iodine or bromine;

Z₁ and Z₂ are independently R3; or O'A'; wherein

R3 is independently selected from the group of alcohols consisting of substituted or unsubstituted linear C₁ to C₈ alkyl groups, substituted or unsubstituted branched C₁ to C₈ alkyl groups and CH₃(CH₂)_nNH₂; wherein n is 0 to 8; or

R₃ is selected from the group consisting of (CH₃)₃N⁺CH₂CH₂OH, HOCH₂CH₂NH₂, HOCH₂CH(NH₂)CO₂H, C₆H₁₁O₆, CH₂OHCHOHCH₂OH; CH₂Ph; and pentoses and hexoses and their corresponding alcohols;

A⁺ is selected from the group consisting of H⁺; K⁺ Na⁺; NH₄⁺; amines selected from the group consisting of mono-, di-, and trialkylamines; and other physiologically acceptable cations;

X is O, S or Se; and

n = 0 or 1.

29. A compound according to Claim 28 that is a 1-O-octadecyl-1,3-propanediol-(C)-phosphonoacetate.

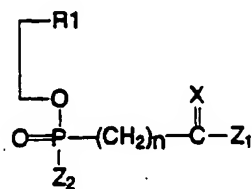
30. A compound according to Claim 28 that is a 1-O-octadecyl-1,3-propanediol-(C)-phosphonoformate.

31. A compound according to Claim 28 that is a 1-O-octadecyl-2-O-benzyl-sn-glycero-3-(C)-phosphonoacetate

32. A compound according to Claim 28 that is a 1-O-octadecyl-2-O-benzyl-sn-glycero-3-(C)-phosphonoformate.

33. A compound according to Claim 28 that is a 1-O-octadecyl-1,2-ethanediol-2-(C)-phosphonoacid.

34. A compound of the formula



wherein

R₁ is an O-alkyl or S-alkyl group, wherein the alkyl group comprises a linear or branched, substituted or unsubstituted C₁ to C₂₄ group, having from 0 to 6 double bonds; or

R₁ is an O-acyl or S-acyl moiety, wherein the acyl group comprises a linear or branched, substituted or unsubstituted C₁ to C₂₄ group, having from 0 to 6 double bonds;

X is O, S or Se;

Z₁ and Z₂ are independently R₃; or O⁻A⁺; wherein

R₃ is independently selected from the group of alcohols consisting of substituted or unsubstituted linear C₁ to C₆ alkyl groups, substituted or unsubstituted branched C₁ to C₆ alkyl groups and CH₂(CH₂)_nNH₂; wherein n is 0 to 8; or

R₃ is selected from the group consisting of (CH₂)₃N⁺CH₂CH₂OH, HOCH₂CH₂NH₂, HOCH₂CH(NH₂)CO₂H, C₆H₁₁O₆, CH₂OHCHOHCH₂OH; CH₂Ph; and pentoses and hexoses and their corresponding alcohols;

A⁺ is selected from the group consisting of H⁺; K⁺ Na⁺; NH₄⁺; amines selected from the group consisting of mono-, di-, and trialkylamines; and other physiologically acceptable cations;

X is O, S or Se; and

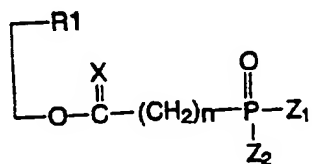
n = 0 or 1.

35. A 1-O-alkyl-1,2-ethanediol-2-phosphonoacid.

36. A 1-O-alkyl-1,2-ethanediol-2-phosphonoacetate.

37. A 1-O-alkyl-1,2-ethanediol-2-phosphonoformate.

38. A compound of the formula



wherein

R₁ is an O-alkyl or S-alkyl group, wherein the alkyl

group comprises a linear or branched, substituted or unsubstituted C₁ to C₆ group, having from 0 to 6 double bonds; or

R₁ is an O-acyl or S-acyl moiety, wherein the acyl group comprises a linear or branched, substituted or unsubstituted C₁ to C₆ group, having from 0 to 6 double bonds;

Z₁ and Z₂ are independently R₃; OR₃ or O'A'; wherein

R₃ is independently selected from the group of alcohols consisting of substituted or unsubstituted linear C₁ to C₆ alkyl groups, substituted or unsubstituted branched C₁ to C₆ alkyl groups and
5 CH₃(CH₂)_nNH₂; wherein n is 0 to 8;

R₃ is selected from the group consisting of (CH₃)₃N⁺CH₂CH₂OH, HOCH₂CH₂NH₂, HOCH₂CH(NH₂)CO₂H, C₆H₁₁O₆, CH₂OHCHOHCH₂OH; CH₂Ph; and pentoses and hexoses and their corresponding alcohols;

10 A' is selected from the group consisting of H⁺; K⁺ Na⁺; NH₄⁺; amines selected from the group consisting of mono-, di-, and trialkylamines; and other physiologically acceptable cations;

X is O, S or Se; and

n = 0 or 1.

39. A 1-O-alkyl-1,2-ethanediol-2(C)-phosphonoacid.

15 40. A 1-O-alkyl-1,2-ethanediol-2(C)-phosphonoacetate.

41. A 1-O-alkyl-1,2-ethanediol-2(C)-phosphonoformate.

20 42. A compound according to any of Claims 1-41 wherein R₁ is an O-alkyl group, and Z₁ and Z₂ are independently R₃, wherein at least one R₃ group is selected from the group consisting of choline, ethanolamine, glycerol and inositol.

43. A liposome formed in part from a compound according to any of Claims 1-42.

44. A pharmaceutical formulation comprising any of the compounds of Claims 1-42.

25 45. A composition containing any of the compounds of Claims 1-42 for use in the treatment of a viral infection.

46. The use of any of the compounds according to Claim 1-42 in the preparation of a medicament for treating a viral or retroviral infection in a mammal.

30 47. A use according to Claim 44 further comprising the incorporation of an antiviral nucleoside analogue, viral protease inhibitor or other antiviral agent into said medicament.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/14940

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE, MEDLINE

search terms: lipids, prodrugs, phosphonoacids, antiviral agents, phosphonoformate, phosphonoacetate, HIV, HSV, HCMV

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
&, P	US, A, 5,411,947 (HOSTETLER ET AL.) 02 May 1995, entire document.	1-47

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	T	later documents published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	A	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
13 FEBRUARY 1996

Date of mailing of the international search report
04 MAR 1996

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer *Deborah Carr*
DEBORAH D. CARR
Telephone No. (703)308-1235

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/14940

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

COTF 9/02

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

554/78,79,80; 558/158

B. FIELDS SEARCHED

Minimum documentation searched

Classification System: U.S.

554/78,79,80; 558/156

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☒ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.